



Higher expression of phosphodiesterase type 5 in the anterior fibromuscular stroma of the human prostate

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Abstract

Purpose To examine phosphodiesterase type 5 (PDE5) expression in the anterior fibromuscular stroma (AFMS) of the prostate. Although PDE5 expression was identified in the human prostate, differences in PDE5 expression in intra-prostatic regions are unknown. The AFMS in the prostate has peculiar innervations that could contribute to voiding function. Here, we examined regional differences in PDE5 expression in the prostate with special reference to the AFMS.

Methods A total 18 human prostate and bladder specimens were obtained. Tissue specimens were processed by hematoxylin–eosin (H&E) staining and immunohistochemistry for PDE5. Immunoreactivity with PDE5 was evaluated using computer-assisted image analysis in the following regions: the AFMS, bladder neck, stromal hyperplasia in the transition zone, glandular hyperplasia in the transition zone (TZ gland), and the peripheral zone (PZ). The correlation between PDE5 expression in the AFMS and clinical data was analysed.

Results Image analysis revealed that the median ratio of the PDE5-immunoreactive area to smooth muscle area by H&E staining was 74.7% in the AFMS. There was significantly higher PDE5 expression in the AFMS than in the TZ gland ($p=0.034$) and PZ ($p=0.002$). PDE5 expression in the AFMS was not significantly correlated with age, prostate volume, transition zone volume, or transition zone index. However, older men had a tendency to have higher PDE5 expression in the AFMS.

Conclusions We found higher PDE5 expression in the AFMS compared with other prostatic regions, which suggested that the AFMS is a target region of PDE5 inhibitors in the prostate.

Keywords Phosphodiesterase type 5 · Anterior fibromuscular stroma · Prostate · Immunohistochemistry

Introduction

Lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH) and erectile dysfunction (ED) are both highly prevalent in elderly men [1]. Phosphodiesterase type 5 (PDE5) inhibitors (PDE5-Is) are the first-line treatment for ED [2]. Randomized placebo-controlled trials have shown that the PDE5-I sildenafil improves voiding and storage LUTS in patients with BPH [3]. PDE5-Is are the standard treatment for BPH and ED.

PDE5 is expressed in the urinary bladder and prostate. A 1998 study cloned human PDE-5A1 cDNA, and PDE5 mRNA expression was identified in the human prostate by

northern blotting analysis [4]. In a 2004 study, PDE5 expression by western blotting analysis was the highest in the prostate among several human tissues including the urinary bladder and corpus cavernosum, and real-time PCR analysis revealed high PDE5 mRNA expression in the prostate [5]. In organ bath studies using human prostate, adrenergic tension in human prostate strip preparations was reversed by sildenafil [6]. PDE5 immunoreactivity was identified in the human bladder [7] and prostate [8, 9], but there are no reports of differences between intra-prostatic regions.

According to McNeal's zonal anatomy [10, 11] (Fig. 1), the human prostate contains a bulk of smooth muscle called the anterior fibromuscular stroma (AFMS), but its physiological function was unknown. In 2001, we reported that the AFMS has significantly different innervations compared with other glandular regions of the prostate [12], and that the AFMS could contribute to age-related urinary disturbance by real-time monitoring of voiding using transrectal ultrasonography (TRUS) [13]. Furthermore, in an ex vivo study,

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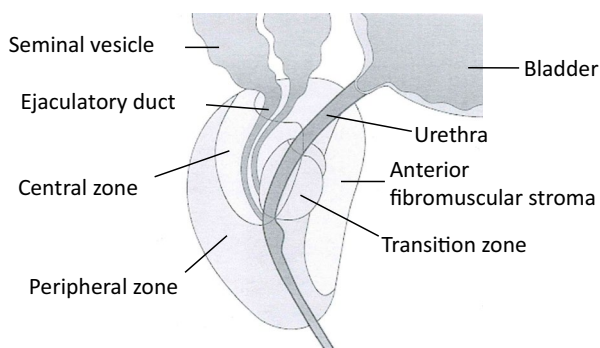


Fig. 1 Zonal anatomy of the prostate (modified from Ref. [11])

division of the AFMS reduced urethral resistance, which suggested that the AFMS has a significant role in maintaining urethral resistance [14]. A magnetic resonance imaging (MRI) study demonstrated that AFMS contraction at initiation of voiding opens the bladder neck, which suggested that the AFMS has an important role in smooth initiation of voiding [15]. According to these reports, the AFMS could be a critical prostate region for voiding function.

Although the expression of PDE5 in human prostate was identified, no study has evaluated zonal differences in PDE5 expression in the prostate. Here, we examined regional differences in PDE5 expression in the prostate with special reference to the AFMS.

Materials and methods

This study protocol was approved by the institutional review boards of Kyoto Prefectural University of Medicine with patient consent.

Tissue sample preparation

A total 18 human prostate and bladder specimens were obtained from patients (55–87 years) who underwent cystoprostatectomy for bladder cancer without prostate infiltration or radical prostatectomy for prostate cancer localized in the peripheral zone (PZ). Immediately after removal, tissue specimens were fixed in 10% buffered formalin and embedded in paraffin wax. Five-micrometer-thick sections were mounted on glass slides and stained with hematoxylin–eosin (H&E) for light microscopy.

Antibody source

Anti-PDE5 antibodies were a generous gift from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Kotera et al. raised the anti-PDE5 antibody and tested the specificity by immunoblotting analysis [16]. The antibody immunoreacted

specifically with recombinant human and rat PDE5 proteins expressed in transfected COS-7 cells and with a native form of PDE5 in extracts of rat platelets, lung, and cerebellum. Immunohistochemical analysis revealed that anti-PDE5 antibody detected immunoreactive material in Purkinje cell layers of the cerebellum, proximal renal tubules, collecting renal ducts, and epithelial cells of pancreatic ducts in rats [16].

PDE5 immunohistochemistry

Paraffin-embedded serial sections were used for H&E staining and PDE5 immunohistochemistry. De-waxed sections were incubated with a 1:50 antibody dilution in phosphate-buffered saline. The specificity of PDE5 immunoreactivity was confirmed in parallel experiments by omitting the primary antibody incubation. Rat cerebellum tissue was used as a positive control as described previously [16]. Selected fields were analyzed from the AFMS, bladder neck (BN), stromal hyperplasia in the transition zone (TZ stroma), glandular hyperplasia in the transition zone (TZ gland), and the PZ. The PDE5 immunoreactive area and smooth muscle area by H&E staining were calculated in each region using a computer-assisted image analysis system (Image J software, National Institute of Health, Bethesda, MD, USA). The ratio of the PDE5-immunoreactive area to smooth muscle area was calculated by computer-assisted image analysis in each region because smooth muscle areas were quite different in each region.

Clinical data

Prostate volume (PV) and transition zone volume (TZV) were calculated using TRUS performed preoperatively. Transition zone index (TZI) was defined as the ratio between TZV and PV in accordance with a previous report [17].

Statistical analysis

Mann–Whitney test was used to compare continuous variables between two regions. Pearson product–moment correlation coefficient was calculated for correlation analysis. A value of $p < 0.05$ was considered statistically significant.

Table 1 Clinical data of 18 men

	Median (IQR)
Age	68 (62–72)
Prostate volume (ml)	24.8 (19.9–28.1)
Transition zone volume (ml)	6.9 (5.7–10.3)
Transition zone index	0.28 (0.24–0.39)

Fig. 2 Immunohistochemistry for phosphodiesterase 5 (PDE5) (a, c, e, g) and hematoxylin–eosin (H&E) staining (b, d, f) in the anterior fibromuscular stroma (AFMS) (a, b), bladder neck (BN) (c, d), peripheral zone (PZ) (e, f), and transition zone gland (TZ) (g). Control sections were assessed by omitting the primary anti-PDE5 antibody and counter-stained with hematoxylin (h). The AFMS and BN had abundant smooth muscle by H&E staining and strong immunoreactive smooth muscle bundles to PDE5. The TZ and PZ had relatively scarce PDE5 immunoreactivity in the stroma (original magnification $\times 100$)

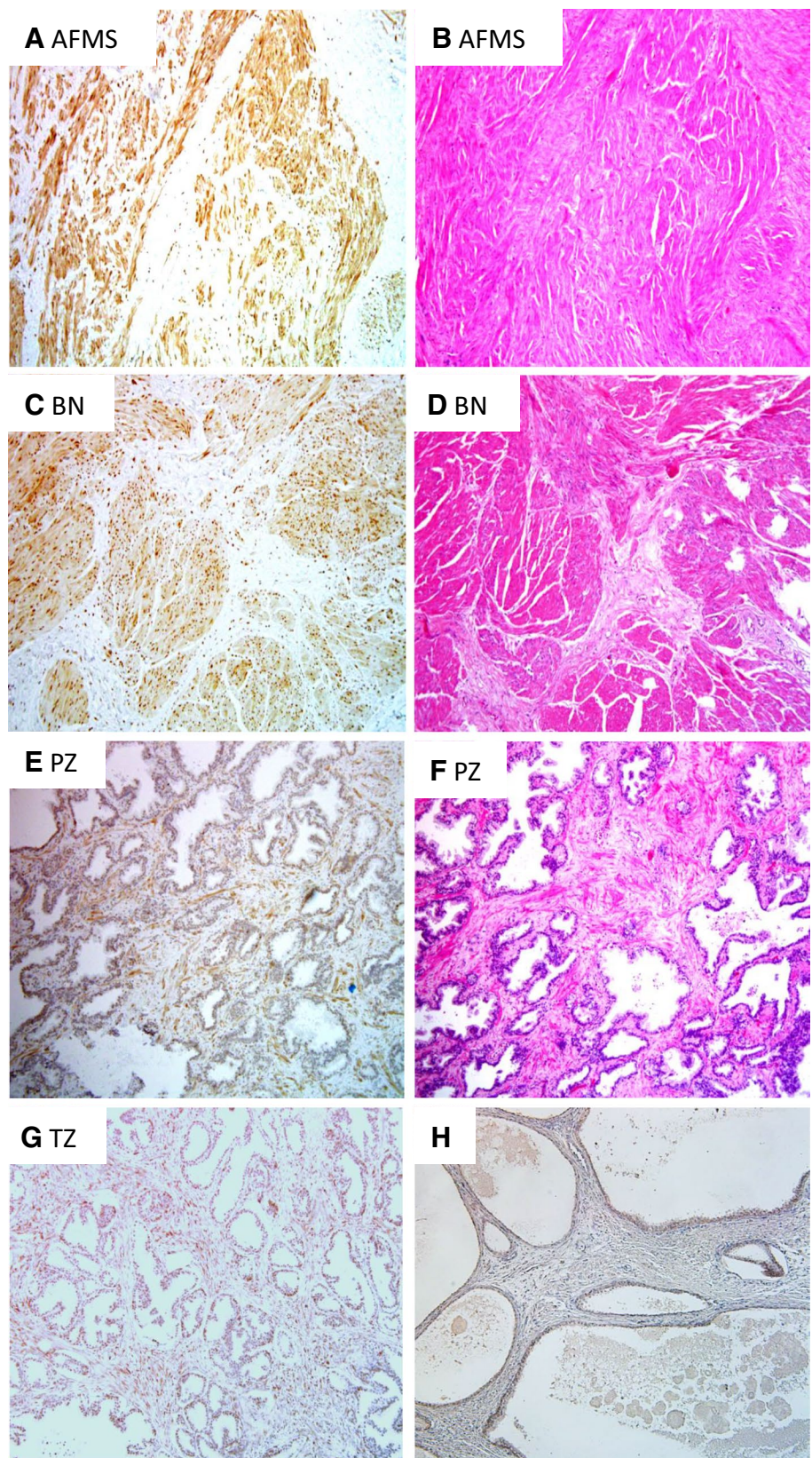


Table 2 The ratio of phosphodiesterase 5-immunoreactive area to smooth muscle area in each region of the prostate

	AFMS (<i>n</i> =18)	BN (<i>n</i> =10)	TZ stroma (<i>n</i> =6)	TZ gland (<i>n</i> =5)	PZ (<i>n</i> =14)
Median percent (IQR)	74.7 (49.1–102.1)	69.4 (40.8–88.7)	36.8 (27.4–82.7)	43.6 (14.1–69.8)	32.2 (25.4–57.9)

Table 3 Statistical analysis between two regions

Regions	<i>p</i> value
AFMS vs BN	0.55
AFMS vs TZ stroma	0.10
AFMS vs TZ gland	0.034
AFMS vs PZ	0.002
BN vs TZ stroma	0.12
BN vs TZ gland	0.24
BN vs PZ	0.02
TZ stroma vs TZ gland	0.78
TZ stroma vs PZ	0.43
TZ gland vs PZ	0.89

AFMS anterior fibromuscular stroma, BN bladder neck, IQR interquartile range, PZ peripheral zone, TZ transition zone, TZ gland glandular hyperplasia in the transition zone, TZ stroma stromal hyperplasia in the transition zone

All statistical analyses were performed using JMP® 14 (SAS Institute Inc., Cary, NC, USA).

Results

Clinical data are shown in Table 1. Median age, PV, TZV, and TZI were 68 years, 24.8 ml, 6.9 ml, and 0.28, respectively.

H&E staining and PDE5 immunohistochemistry images are shown in Fig. 2. The AFMS and BN had abundant smooth muscle by H&E staining and strong immunoreactivity to PDE5 in smooth muscle bundles. The TZ and PZ had relatively scarce PDE5 immunoreactivity in the stroma.

Image analysis revealed that the median ratio of the PDE5-immunoreactive area to smooth muscle area by H&E staining was 74.7% in the AFMS, 69.4% in the BN, 36.8% in the TZ stroma, 43.6% in the TZ gland, and 32.2% in the PZ (Table 2). Statistical analysis between the two regions showed significantly higher PDE5 expression in the AFMS than in the TZ gland ($p=0.034$) and PZ ($p=0.002$) (Table 3). PDE5 expression in the AFMS was higher than in the BN and TZ stroma, but the difference was not significant. The BN had higher PDE5 expression than the PZ ($p=0.02$).

The ratio of PDE5-immunoreactive area to smooth muscle area in the AFMS was not significantly correlated with age, PV, TZV, or TZI. However, older men had a tendency to have higher PDE5 expression in the AFMS ($r=0.466$, $p=0.051$) (Fig. 3).

Discussion

We found higher expression of PDE5 in the AFMS compared with other prostatic regions using immunohistochemistry. There have been no reports examining differences in PDE5 expression among intra-prostatic regions, especially the AFMS. This is the first report to analyze PDE5 expression in the AFMS.

There have been a few reports on the expression of PDE5 in the TZ of the prostate. Upregulation of PDE5 occurs in human hyperplastic prostate stroma, which may explain the effectiveness of PDE5-Is for treating LUTS and BPH [18]. The authors collected prostate tissue from cystoprostatectomy patients with BPH. Although they did not describe which zone they collected the tissue from, the analyzed prostate tissue would be mainly from TZ. Zhao et al. [19] in a cohort of male patients presenting with moderate-to-severe LUTS, to whom a clinical dosage of either the PDE5-I tadalafil or udenafil was administered 1 h prior to transurethral resection of the prostate, demonstrating that both drugs significantly increased cyclic guanosine monophosphate (cGMP) levels in the prostate tissue. It is considered that prostate tissue was mainly from the TZ in this article. An immunohistochemical analysis using TZ tissue showed that PDE5 was localized in close conjunction to key mediators of the nitric oxide (NO)/cGMP pathway [20]. These previous studies indicated that the TZ could be a target lesion for PDE5-Is.

These previous articles did not investigate intra-prostate zonal differences. We analyzed the expression of PDE5 especially focusing on the AFMS based on McNeal's zonal anatomy of the prostate in comparison with other regions of the prostate. To the best of our knowledge, there have not been any reports regarding the expression of PDE5 in the AFMS. This is a strength of our study. We identified higher expression of PDE5 in the AFMS compared with other regions. Our results suggested that not only the TZ, but also the AFMS may be a target region to PDE5-Is.

In a review article on PDE5-Is, the authors proposed that improvement in LUTS by tadalafil may be caused by smooth muscle cell relaxation in the BN, prostate, and urethra, with the maintenance of effect possibly supported by smooth muscle cell relaxation in these organs' vascular supply, and increased blood perfusion and oxygenation [21]. Our results demonstrated that especially the AFMS in the prostate may have an important role with PDE5-Is on LUTS. Our previous study suggested that innervations in the AFMS

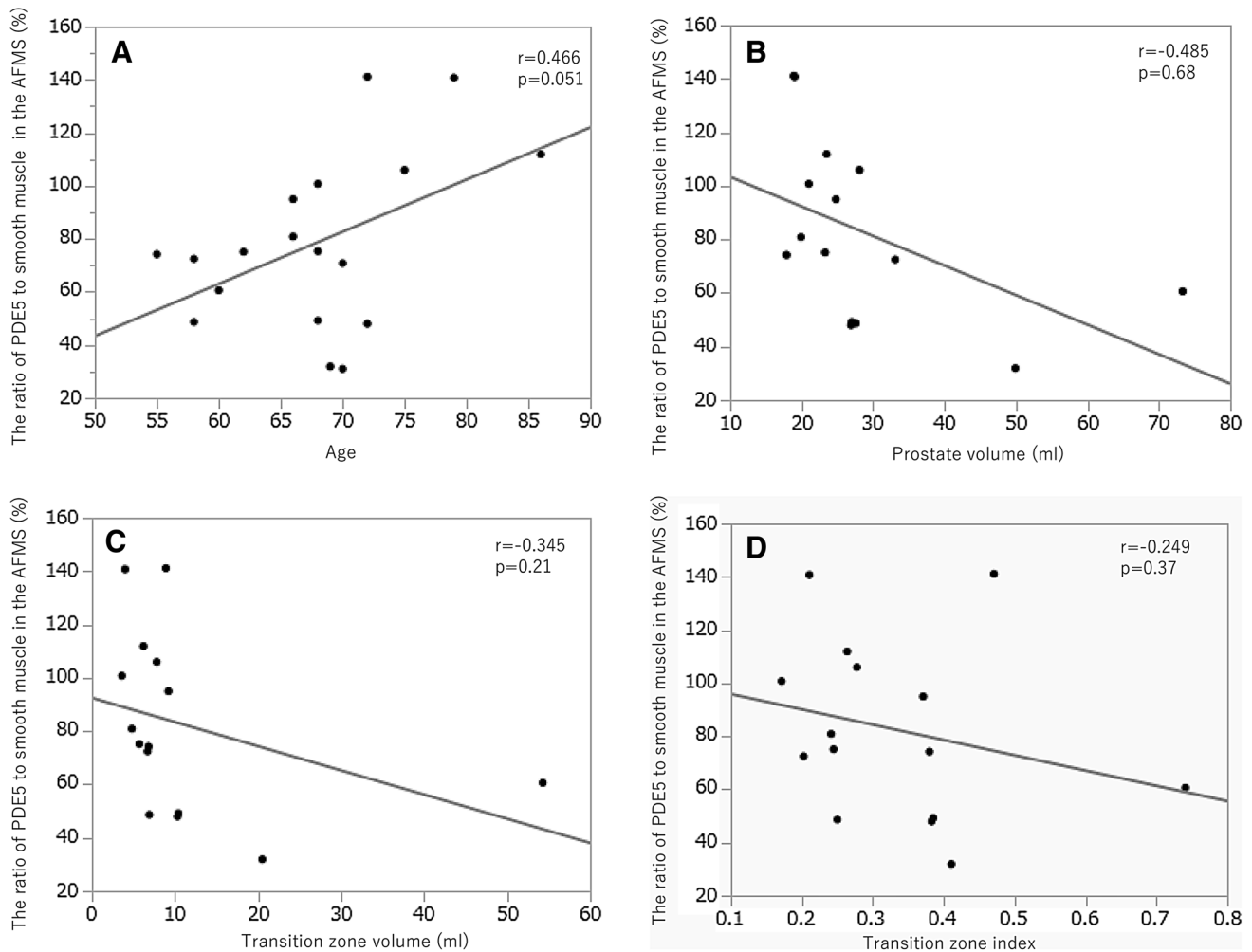


Fig. 3 Correlation analysis between the ratio of phosphodiesterase 5 (PDE5)-immunoreactive area to smooth muscle area in the anterior fibromuscular stroma (AFMS), and age (a), prostate volume (PV) (b), transition zone volume (TZV) (c) and transition zone index (TZI) (d).

may be similar to that of the BN, and synchronizing the AFMS and the BN may open the prostatic urethra during micturition [12]. We found abundant smooth muscle and PDE5 expression in the AFMS and BN, which supports this theory. PDE5-Is could have direct functions in the BN and the AFMS by opening the BN and prostatic urethra during micturition. Furthermore, the AFMS could actively contribute to smooth voiding from clinical research by TRUS [13] and MRI [15] during micturition.

We hypothesized that there might be a correlation between PDE5 expression in the AFMS and the degree of prostatic enlargement. The immunoreactive area of PDE5 in the AFMS was not correlated with PV, TZV, or TZI in this study, although PV and TZV were not especially high; the median PV and TZV were 24.8 and 6.9 ml, respectively. These results meant that many patients did not have severe prostatic enlargement in this study. Therefore, our

The ratio of PDE5-immunoreactive area to smooth muscle area in the AFMS was not significantly correlated with age, PV, TZV, and TZI. However, older men had a tendency to have higher PDE5 expression in the AFMS ($r=0.466$, $p=0.051$)

results may be restricted to patients without severe prostatic enlargement. More patients with advanced BPH should be included to fully analyze the correlation between PDE5 expression in the AFMS and PV.

There may be some controversy in distinguishing each region. We were able to distinguish each region by observing whole sections of the prostate to clarify the zonal anatomy [10]. The AFMS was identified in the anterior portion of the prostate and consisted of non-glandular tissue. The TZ and PZ were identified by their anatomical relation to the urethra and ejaculatory duct, which are good markers to detect each region in the prostate. The investigated fields in each region were selected from the definitively identified regions.

PDE5 was expressed in the prostatic stroma [9], and the endothelium and smooth muscle cells of blood vessels [22]. In this study, we analyzed the PDE5 total immunoreactive area regardless of the histological portions because the area

of smooth muscle was much larger than the area of blood vessels. The expression of PDE5 in the endothelium of blood vessels might be different from that in smooth muscle.

Metabolic factors are important for both prostate inflammation and enlargement in men with LUTS [23]. PDE5-Is may reduce inflammation associated with fibrosis, improve oxygenation, and normalize prostate structural anatomy and physiological activity [24, 25]. Because the AFMS becomes fibrotic during aging [26], the AFMS could be a region within the prostate for PDE5-Is to relieve LUTS.

PDE 5 expression in the AFMS was not significantly correlated with age. However, older men had a tendency to have higher PDE5 expression in the AFMS. This finding may suggest that age-related urinary disturbance is related to higher PDE5 expression in the AFMS.

Our study had several limitations. (1) The number of samples was limited. (2) Many patients did not have severe prostatic enlargement and our results may be restricted to the patients without severe prostatic enlargement. (3) All specimens were from men over 55 years of age. Younger specimens should be analyzed to evaluate age variation in PDE5 expression in the AFMS, and our conclusions are limited to older men. (4) An immunohistochemical study using TZ tissue showed that PDE5 was co-localized with cGMP, cGMP-binding protein kinase type 1, and cGMP-binding protein kinase A, which are key mediators of the NO/cGMP pathway [20]. In our study, co-localization of PDE5 with these key mediators was not evaluated. The co-localization of PDE5 with other key mediators should be examined to further clarify the possible function of the AFMS to PDE5.

Conclusions

We found higher PDE5 expression in the AFMS compared with other prostatic regions, which suggested that the AFMS could be a target region of PDE5-Is.

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